Diversity of Coronaviruses in Bats: Insights into Origin of SARS Coronavirus

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Background: Although the finding of SARS coronavirus (SARS-CoV) in caged palm civets suggested that wild animals are the origin of SARS-CoV, subsequent studies suggested that civet may have served only as an amplification host. In 2005, we identified a coronavirus closely related to SARS-CoV (bat-SARS-CoV) in Chinese horseshoe bats. However, it remains to be determined if bat-SARS-CoV or other coronaviruses in bats are the direct progenitor of SARS-CoV.

Methods: To understand the diversity and evolution of coronaviruses in bats, a 2-year surveillance study for coronaviruses was conducted in bats from various rural areas in Hong Kong. As coronaviruses are known to have high recombination frequency, the genomes of the identified novel coronaviruses were also sequenced and analyzed to determine possible recombination events responsible for interspecies transmission.

Results: Among 1389 bats of 16 species from 24 different locations, coronaviruses were identified from anal swabs of 132 (9.5%) bats by RT-PCR. Phylogenetic analysis revealed at least seven novel coronaviruses from seven different bat species, in addition to bat-SARS-CoV. Five of them belonged to group 1 coronaviruses while two belonged to group 2 coronaviruses. Besides bat-SARS-CoV, Chinese horseshoe bats were found to harbor another novel group 1 coronavirus. The genome of this virus represents the smallest coronavirus genome and possessed a unique spike protein evolutionarily distinct from the rest of the genome and containing a 15-amino acid peptide homologous to a corresponding peptide within the RBM of spike protein of SARS-CoV, suggesting a common evolutionary origin in the spike protein of this group 1 bat coronavirus, bat-SARS-CoV, and SARS-CoV.

Conclusion: Bats are important reservoir for a huge diversity of coronaviruses, including SARS-CoV-like viruses. The finding of another group 1 coronavirus in Chinese horseshoe bats with a homologous peptide to SARS-CoV warrants further investigations on the origin of the SARS-CoV spike protein.

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14.006

Analysis of Putative Virulence Factors in *Enterococcus faecium* Isolated from Different Sources in Iran

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Background: Enterococci have emerged as a leading cause of nosocomial infections. The potential virulence factors include a cytolytic toxin, gelatinase, an aggregation substance, the enterococcal surface protein (Esp) and infections is still limited.

Methods: A total of 98 strains containing, 49 clinical Vancomycin-resistant *Enterococcus faecium* (cl-VREFm) and 49 environmental VREFm (en-VREFm), were included in this study. The phenotypical characterization was performed by their abilities to adhere to Vero cell line and to associate their phenotypes with the presence of known virulence genes detected within their genomes by PCR. The following genes were amplified by PCR: asa1 (aggregation substance), cyl A, B, M (cytolysin), hyl (hyaluronidase), gelE (gelatinase) and esp (enterococcal surface protein). The transferability of esp gene were examined by filter mating tests. The strains were typed by Pulsed-field gel electrophoresis.

Results: The genes that encode cytolysin and aggregation substance was never detected in isolates, whereas gelE and esp genes was detected in both clinical and environmental strains and hyl gene was only detected in clinical isolates (28%).The following data were obtained in this study: 81% of cl-VREFm and 80% of en-VREFm isolates were positive for esp gene, the gelE gene was detected in all of clinical and 47% of environmental isolates. Strong adhesion was observed only in clinical strains. None of the esp genes were transferable by conjugation tests. According to PFGE results the isolates were heterogeneous.

Conclusion: Environmental isolates were equipped with fewer virulence factors than clinical isolates and presence of virulence factors in environmental isolates demonstrates that they can be potentially virulent for human. There was a correlation between PCR and phenotypic tests in clinical strains. Phenotypic testing revealed the existence of apparently silent gelE and esp genes among environmental strains.

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14.007

Immunogenicity of Novel Consensus-Based DNA Vaccines Against Chikungunya Virus

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CHIKV is an emerging arbovirus and is an important human pathogen that causes a syndrome characterized by fever, headache, rash, nausea, vomiting, myalgia, arthralgia and occasionally neurological manifestations such as acute limb weakness. It is also associated with a fatal haemorrhagic condition. CHIKV is geographically distributed from Africa through Southeast Asia and South America, and its transmission to humans is mainly through Aedes species mosquitoes. The frequency of recent epidemics in the Indian Ocean islands suggests that something else was carrying the virus, as Aedes aegypti are not found there. In fact, the relative Asian tiger mosquito, Aedes albopictus, was present and has raised concern in the world health community regarding the

potential for a CHIK virus pandemic. Efforts to monitor the disease will only provide minimal warning in a global society, and steps must be taken to prevent the morbidity and mortality associated with a possible pandemic. There is no specific treatment for Chikungunya virus and there is no vaccine currently available. We propose a novel consensus-based approach to vaccine development, employing a DNA vaccine strategy that can provide more highly cross-reactive cellular immunity against CHIK virus. The vaccine cassette was designed based on Capsid (Cap) and Envelope (E1) specific consensus sequences with several modifications, including codon optimization, RNA optimization, the addition of a Kozak sequence, and a substituted immunoglobulin E leader sequence. The expression of Cap and envelope E1 was evaluated using T7-coupled transcription/translation and immuno blot analysis. A recently developed, adaptive constant-current electroporation technique was used to immunize mice (both Balb/C & C57BL/6 mice strain) with an intramuscular injection of plasmid coding for the CHICK-Cap and E1. We show such constructs can induce strong cellular immunity against CHIK-Cap and E1 antigens. The analysis of specific antibody responses suggested that CHIK-E1 could induce a strong E1 specific antibody response. Epitope mapping results indicated that there is an increase in the breadth and magnitude of cross-reactive cellular responses induced by both the Capsid and Envelope immunogen. These properties suggest that such a consensus immunogen deserves further examination for its potential to serve as a component antigen in a CHIK vaccine cocktail.

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14.008

Post-Exposure Vaccination with a Highly Attenuated Vaccinia Vaccine, LC16m8, for Protection of Nonhuman Primates from Monkeypox

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Introduction: A highly attenuated smallpox vaccine, LC16m8, is generated from parent strain, *vaccinia virus Lister*, by passages of Lister in primary rabbit kidney cells at a temperature of 30 °C. LC16m8 lacks expression of the full-length B5R membrane protein, one of the most important viral membrane proteins for induction of immune response to vaccinia virus, due to a frameshift mutation in the gene. A single vaccination with LC16m8 protects non-human primates (NHPs) from monkeypox, monkeypox virus infection. In the present study, the efficacy of LC16m8 as a therapeutic vaccine was evaluated in protection of NHPs from monkeypox.

Materials and Methods: Twenty-two NHPs (*Macaca fascicularis*) were used. The NHPs were immunized with LC16m8 or mock followed by subcutaneous inoculation of monkeypox Zr-599. Three were mock-immunized and infected with the virus. Three were infected with the virus and then immunized with LC16m8. Three, 3, and 5 NHPs were infected with the virus 3, 7, and 14—24 days after vaccination, respectively. Clinical manifestations were monitored. Viremia level and antibody response were determined by the quantitative real-time PCR and IgG-ELISA, respectively. Pathological examination was carried out in these subjects.

Results: Post-exposure vaccination with LC16m8 for NHPs, which were infected with monkeypox virus, improved the clinical manifestation of monkeypox, while all the naive subjects were nearly lethal. Vaccination with LC16m8 7 days before monkeypox virus challenge completely protected NHPs from monkeypox. Viremia level in the post-exposure vaccinated subjects was significantly lower than that in the naive subjects.

Discussion: LC16m8 has been re-produced and stockpiled in Japan for the possible threat of bioterrorism with variola virus as a bioweapon. Based on these results, it is suggested that LC16m8 may protect humans from smallpox, if they were immunized with LC16m8 immediately after the event of variola virus infection.

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14.009

Distribution and Genetic Diversity of *Plasmodium falciparum* Erythrocyte Binding Antigen 175 and Clinical Outcome of Malaria in the Kassena-Nankana District

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The Erythrocyte Binding Antigen 175 (EBA 175) is a 175 kDalton *Plasmodium falciparum* antigen which plays a major role in erythrocyte recognition by the parasite. It also induces antibodies which inhibit merozoite invasion. EBA 175 has been sequenced from FCR-3 and CAMP strains of *Plasmodium falciparum*. The sequences were identical in most parts of the gene, differences were apparent in the 423bp segment in the FCR-3 strain, the F-genotype, and the 342bp segment, the C-genotype. Parasite strains possess either one or the other segment and never both. The functions and potential effects of this dimorphism remain unclear. This study therefore investigated the relationship between this dimorphism and clinical outcome of malaria. A nested polymerase chain reaction (PCR) was used to determine the genotypes of the parasite strains that exhibit this dimorphism in severe, mild and healthy controls of malaria from the Kassena Nankana District (KND), an area which had been earmarked for future vaccine trial. A total of 299 samples were analysed, 232 of these samples were positive with *Plasmodium falciparum* infections, these comprised 75 (32.2%), 76 (32.6%) and 81 (35.5%) samples of severe, mild and healthy controls of malaria respectively, were genotyped for the EBA-175 gene. The severe samples, had a distribution of 44 (58.66%), 24 (32%) and 7 (9.3%) of the F, C and CF EBA-175 genes respectively; the mild samples comprised, 42 (55.3%), 29 (38%), and 5 (6.6%) of the F, C and CF EBA-175 genes respectively; the healthy controls: 59 (72%), 21 (26%), and 13 (16%) of the F, C and CF EBA-175 genes respectively. A chi-square test revealed that the mixed genotype (CF) is significantly associated with severe malaria ($p = 0.04$; OR = 8.23, 95% CI = 1.048—64.7), whereas